Review

BARD1 and breast cancer: the possibility of creating screening tests and new preventive and therapeutic pathways for predisposed women

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Abstract: Current oncological developments are based on improved understanding of genetics, and especially the discovery of genes whose alterations affect cell functions with consequences for the whole body. Our work is focused on the one of these genes, the BARD1 and its oncogenic role in breast cancer. Most importantly, the study points to new avenues in the treatment and prevention of the most frequent female cancer based on BARD1 research. The BARD1 and BRCA1 proteins have similar structures and functions, and they combine to form the new molecule BARD1-BRCA1 heterodimer. The BARD1-BRCA1 complex is involved in genetic stabilization at the cellular level. It allows to mark abnormal DNA fragments by attaching ubiquitin to them. In addition, it blocks (by ubiquitination of RNA polymerase II) the transcription of damaged DNA. Ubiquitination, as well as stabilizing chromatin, or regulating the number of centrosomes, confirms the protective cooperation of BARD1 and BRCA1 in the stabilization of the genome. The overexpression of the oncogenic isoforms BARD1β and BARD1δ permit cancer development. The introduction of routine tests, for instance, to identify the presence of the BARD1β isoform, would make it possible to detect patients at high risk of developing cancer. On the other hand, introducing BARD1δ isoform blocking therapy, which would reduce estrogen sensitivity, may be a new line of cancer therapy with potential to modulate responses to existing treatments. It is possible that the BARD 1 gene offers new hope for improving breast cancer therapy.

Keywords: breast cancer; BARD1; surveillance; management; genetic testing; predisposition; susceptibility; neoadjuvant; chemotherapy

1. Introduction

In 1996, Wu et al. discovered a binding partner protein of BRCA1 (BReast CAncer type 1) which they named BRCA1-associated RING domain 1 (BARD1) [l]. The BARD1 gene is located on chromosome 2 and encoded by the sequence 2q34-q35. Its product is a 777 amino acid protein composed of an N-terminal RING-finger domain, three Ankyrin repeats (ANK) domains and two tandem BRCA1 C-terminal (BRCT) domains (Figure 1). The BARD1 protein structure is like that of the BRCA1, however it is different from that of the BRCA2 (BReast CAncer type 2), the second gene associated with breast cancer [2]. BARD1 and BRCA1 can form a heterodimer by their N-terminal

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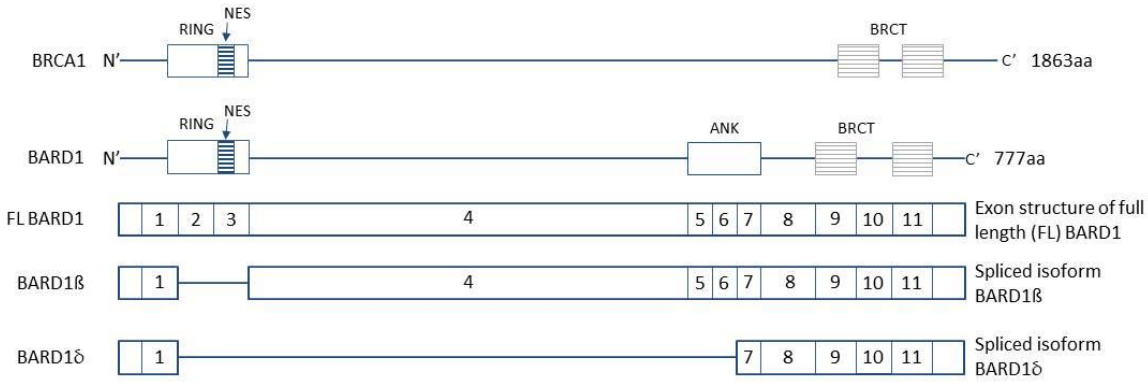
RING finger domains which form a stable complex [3]. The full length-BARD1 (FL-BARD1) protein has tumor-suppressor functions whether it acts as a heterodimer or in BRCA1 independent pathways. However, the aberrant splice variants of BARD1 have oncogenic functions. The two major isoforms involved in the breast cancer pathogenesis are BARD1β and BARD1δ [4].

Figure 1. Schematic structures of BRCA1, BARD1 and isoforms: BARD1β, BARD1δ.



Legend: RING finger domains enables to form stable complex between BRCA1 and BARD1. NES are nuclear export signals which together with NLS (nuclear localization signals) are necessary for proper intracellular localization of BARD1. ANK (Ankyrin repeats) interacts with several proteins including p53 and NF-κB. BRCT (BRCA1 carboxy-terminal domain) motifs fold into a binding pocket with a key lysine residue (K619).

The BARD1-BRCA1 heterodimer, as an E3 ubiquitin ligase, is essential in numerous cell regulations [4]. Its primary function is to allow ubiquitin to be attached to different proteins which marks them for further degradation. Due to this ability, the BARD1-BRCA1 heterodimer is engaged in the DNA damage response pathway [4]. Its BRCT motifs are phosphoprotein-binding modules and bind to poly(ADP-ribose) (PAR), which targets the BARD1-BRCA1 heterodimer to DNA damage sites, where it acts as an E3 ubiquitin ligase. As a result, BRCA1 is able to participate in all major DNA repair pathways [5,6].

Moreover, this heterodimer prevents the transcription of the damaged DNA and maintains its genetic stability by ubiquitinating RNA polymerase II [7].

The BARD1-BRCA1 heterodimer is also responsible for the ubiquitination and subsequent degradation of estrogen receptors alpha (ERα). It is an important function in terms of pathogenesis of breast cancer as estrogen receptors alpha (ERα) and beta (ERβ) activate genes responsible for cell proliferation. [8].

BARD1 is also able to function as a protein in the BRCA1-independent pathways. BARD1 has a crucial role during the induction of apoptosis by the stabilization of p53 [9]. Likewise, it inhibits mRNA maturation during genotoxic stress through having an impact on CstF-50 (cleavage stimulation factor) [10].

All these functions prove that FL-BARD1 has an important tumor suppressor role. However, in neoplastic pathogenesis, BARD1 isoforms antagonize FL-BARD1 and enable uncontrolled proliferation. The main cancerous isoforms are BARD1β and BARD1δ.

The first of isoforms, BARD1β, stabilizes Aurora kinases A and B. It forms a complex with BRCA2 and Aurora B during telophase and cytokinesis that results in overriding the mitotic checkpoint and excessive cell proliferation. Thus, Aurora family of kinases and BARD1β expression levels might be predictive biomarkers for responses to Aurora inhibitors [11]. The second key isoform,

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BARD1δ, interacts with ERα and antagonizes FL-BARD1 that results in a higher response rate to estrogens [8].

Breast cancer is the second most common neoplasia in the female population. Despite this fact, no more than 40% of familial breast cancers have been identified as having causative gene mutations [12]. Most of these mutations are in either the BRCA1 or the BRCA2 genes. The latest reports show though that deleterious BARD1 variants may be the reason for hereditary breast cancer in BRCA1 and BRCA2 negative families [13]. There are already available new types of tests that show the presence of mutations not only in the BRCA1 or BRCA2 gene, but also in BARD1. BARD1 seems to be an interesting target for novel therapies as it is involved in many different cellular processes and therefore it has a lot of potential therapeutic targets.

The BARD1 protein also seems to be an interesting starting point in analyzing the causes of drug resistance in breast cancer cases. About 70% of breast cancers are ER positive. Despite using multiple drugs that are ER antagonists (e.g., tamoxifen) we still observe numerous relapses, even during 15 years of post-treatment follow-up [14]. The main limitation in solving this problem is that the mechanisms of chemoresistance are still too-little understood. However, it seems that the BARD1 protein, that is associated with so many cellular mechanisms, can play a key role here [14].

The aim of our review is to investigate the role of the BARD1 gene in the assessment of predisposition to breast cancer, which is related to the question of the usefulness of testing this gene in screening programs for families with familial history of breast cancer, and further - to investigate it as a potential target of new anticancer therapies, including sensitivity to chemotherapy.

2. Scope of the review

The article reviews the literature using the Pubmed, Google Scholar and Elsevier Clinical Key databases using the terms: "breast cancer"; "BARD1"; "surveillance"; "management"; "genetic testing"; "predisposition"; "susceptibility"; "neoadjuvant"; "chemotherapy" in various combinations as appropriate. Articles were screened for relevance, those with the most up-to-date information were selected for inclusion. In addition, a manual search of the reference lists of previously captured articles was carried out to increase the likelihood of choosing essential studies.

3. The results of the review on the topics covered

We have focused on four main issues regarding BARD1. The first one discusses the frequency of mutations in the BARD1 gene in non-BRCA1 and non-BRCA2 patients with breast cancer. It shows that BARD1 is one of the most common non-BRCA1/2 genes to mutate. For this reason, subsequently we present different possibilities for running a surveillance program for BARD1 for example detection of BARD1 gene isoforms by using specific antibodies or radiogenomics, which link clinical assessment with imaging results and genetic background. The next part discusses the possibility of using BARD1 as a target for new therapies using drugs such as an inhibitor of CDKs, mTOR inhibitor, PI3K inhibitors or PARP inhibitors (inhibitors of the enzyme poly ADP ribose polymerase) and a one of the histone deacetylase inhibitors. Finally, we consider BARD1 gene mutations and neoadjuvant setting in breast cancer which is important medical treatment modality for breast cancer patients treated today.

3.1. The significance of BARD1 in genetic predisposition to breast cancer

Genetic predisposition to breast cancer can be divided into three different levels [15,16], depending on the risk of breast cancer and the degree of gene penetrance. The first level is comprised of high-risk heterozygous, and highly penetrant gene mutations. The second level is associated with genes of intermediate penetrance and a moderate risk of breast cancer. The third level consists of low-penetrance breast cancer susceptibility alleles, and common polymorphisms (SNPs - single nucleotide polymorphisms) [13,16] (Table 1).

Since its discovery in 1996, the BARD1 gene and its various mutations have been extensively studied for breast cancer susceptibility. In a study of over 65,000 American non-BRCA1 and non-

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BRCA2 patients (mean age at diagnosis 48.5) with breast cancer, pathogenic variants in BARD1 in white women were associated with a significant moderately increased risk of breast cancer. The pathogenic variant (PV) in this population, proved to be quite rare (<1 out of 500 breast cancer cases) [20].

Table 1. Levels and characteristic of genetic predisposition to breast cancer.

Level of predisposition	Gene penetration	Risk of breast cancer	Examples of affected genes	Characteristics	Reference
I	High	High	BRCA1 and BRCA2, TP53, CDH1, STK11, PTEN	Mutations in BRCA1 and BRCA2 are responsible for 16-40% of hereditary breast and ovarian cancers and site-specific breast cancer; inTP53 is associated with up to 85% risk of developing breast cancer by age 60; germline mutations in CDH1 and STK11 are associated with 39-52% and 32-54% risk of developing breast cancer, respectively; germline mutations in the PTEN gene promoter are associated with an 85% lifetime risk of breast cancer	[12,17,18,19]
II	Intermediate	Moderate	ATM, CHEK2, BRIP1, BARD1, PALB2	Mutations in these genes are responsible for a 2- to 4-fold increase in the risk of breast cancer in comparison to population-based risk	[16]
III	Low	Low	FGFR2, RAD51	FGFR2 SNPs increase the risk of breast cancer by increasing the response to estrogen; RAD51 SNP2 i.e. are considered as BRCA1/2 mutations carriers risk modifiers	[13,16]

BARD1 is not only thought to be a breast cancer susceptibility gene, but also a gene predisposing to triple negative breast cancer (TNBC) [21]. Furthermore, in a study of 10,901 TNBC patients, it was established that BARD1 was one of the most common non-BRCA1/2 genes to mutate. Among other genes [21], BARD1 was proven to be statistically significantly associated with a moderate to high risk of TNBC with an incidence of 0.5-0.7% [21]. The same study established that the PVs in BARD1 were

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associated with a lifetime risk of TNBC in 7% of cases; and a 21% risk for Caucasian patients and 39% risk of TNBC for African American patients [21]. In a different study of 289 African American patients, 144 of whom were cases of familial breast cancer, only 1 incidence of PV in the BARD1 gene was found [22]. In another study of 1,824 female American patients with TNBC, 97% of which were white, 1.9% African, 0.6% Asian and 0.6% Hispanic, deleterious mutations in BARD1 were detected 9 times, with an incidence of 0.3-0.5% [23].

Outside the United States, there has also been researched on BARD1 in Europe, Korea, and Australia. Out of 120 Korean breast cancer patients negative for BRCA1/2 mutations, PVs in the BARD1 gene were identified in two patients [24]. A Finnish study of 94 BRCA1/2 negative breast cancer families, established an incidence of 7.4% of Cys557Ser allele in the BARD1 gene in comparison with an incidence of 1.4% in the healthy controls [25]. Moreover, the BARD1 Cys557Ser allele was also reported in an Italian study with an incidence of 2.5% [26]. These studies may indicate that the BARD1 Cys557Ser allele is of European origin.

In three independent studies of the Polish population, a deleterious nonsense pathogenic BARD1 mutation, namely p.Q564X, was identified [13,27,28]. A study among 12,476 Polish and 1,459 Belarusian breast cancer patients, identified a 0.27% incidence of the PV in both study groups, assessing it as a low/moderate breast cancer predisposition gene. The p.Q564X BARD1 mutation is possibly a founder mutation, present at least in Central Europe. However, its presence in the Polish control subgroup (0.15%) might indicate its low penetrance. It is also important to point out, that a higher incidence of the mutation was found in progesterone receptor-negative breast cancer patients than in the group of receptor-positive breast cancer patients (0.55% and 0.24%, respectively) [29]. An analysis of large mutations of the BARD1 gene in 504 breast cancer/ovarian cancer Polish patients was conducted and indicated that such mutations do not contribute to breast cancer predisposition [13]. This, however, does not contradict the role of BARD1 as a breast cancer susceptibility gene.

A study in Germany, inspecting germline loss-of function (LoF) variants in the BARD1 gene, was conducted in 4,469 breast cancer patients, 23 (0.51%) of whom had LoF variants. Those patients were significantly younger at first diagnosis than in the overall population sample (median age 42.3 vs 48.6, respectively). LoF BARD1 variants were not significantly associated with patients with age at first diagnosis of equal or older than 50 years. This might suggest a need to intensify breast cancer surveillance programs and include testing for BARD1 PV [30].

However, controversy remains as to whether the BARD1 variant, in its rarity, can be clinically associated with increased breast cancer risk [24]. There have also been studies disputing that BARD1 is a moderate/high-risk breast cancer susceptibility gene [31]. In a study of 684 Australian non-BRCA1/2 patients with familial breast cancer, 4 cases of PVs in BARD1 were identified (0.6%), and the study concluded there is no clinical value for the BARD1 PV mutation testing in breast cancer families.

3.2. Utility of BARD1 in surveillance programs

Currently, BRCA1/BRCA2 is the best-known gene relating to breast cancer. Depending on their age, those carriers at high, or very high-risk need: regular breast self-examination, imaging such as mammography or breast magnetic resonance imaging (MRI) every 6 to 12 months, transvaginal ultrasound every 6 months, and CA-125 blood testing due to the increased risk of ovarian cancer [32,33]. Patient monitoring can also include prophylactic mastectomy and bilateral salpingo-oophorectomy; though these procedures severely affect the patient's quality of life and can hamper her psychosocial well-being as a result of infertility [34,35]. Bearing this in mind, it seems justified that stricter monitoring should be undertaken, including risk stratification based on genetic testing. The BARD1 gene appears beneficial for patient observation. Based on 2019 study of a group of 4469 women, it was concluded that the BARD1 gene correlates with early onset of breast cancer and a worse prognosis [30,36]. The mutated gene carriers should be screened at a younger age, especially because the gene has also been shown to be related to other cancers, including ovarian cancer, colorectal cancer, non-small-cell lung carcinoma and hepatocellular carcinoma [37,38]. The breast cancer cells produce isoforms of the BARD1 gene, which can be detected with specific antibodies

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[39,40,41]. Interestingly, the isoforms can also be produced by spontaneous breast cancer not associated with BRCA group genes [42]. The above phenomenon may correlate with the fact that BARD1 functions as a factor of apoptosis, unrelated to BRCA1. Isoforms excessively expressed in tumor cells do not have suppressor functions, which leads to faster progression and poor prognosis [43,44,4].

A study published in 2007 found BARD1 isoforms to have a distinctive expression pattern. The full-length isoform accounted for 0%, while splice isoforms associated with alternative transcription initiation in exon 4 - for as much as 80.8% in different breast cancer cell lines (21 out of 26) [39].

In addition to the most obvious role of a screening test, the antibody testing can also be used for treatment monitoring because the increased expression of BARD1 isoforms is associated with disease progression. Immunohistochemical testing of breast cancer samples shows more intense staining of the cytoplasm due to the overexpression of BARD1 isoforms. It is worth mentioning that the degree of staining was proportional to the degree of malignancy and size of the tumor. Comparing those observations with the Tumor-Nodes-Metastasis staging system, it is hypothesized that overexpression of BARD1 isoforms is proportional to the size of the tumor and its malignancy grade, which in turn heralds a worse prognosis [44,45,46]. Recently, there has been a growing body of research suggesting that the BARD1 gene is only associated with low to intermediate risks of breast cancer [29]. Other genes, such as PALB2, BRIP1, ATM, CHEK2, RAD51C, RAD51D, NBN, NF1, and MMR should also be considered, as these can have a cumulative effect on the risk of breast cancer in combination with the BARD1 gene [19,46]. Our observations made here strongly advocates for patient surveillance based on multigenetic panel testing, or even for an individualized approach and monitoring based on genetic profiling [47].

Mammography and breast MRI remain the fundamental imaging modalities for the high and very high-risk patients. Decreasing mortality rates are thought to have resulted from more effective treatment [48]. Therefore, other diagnostic tools should be sought, not only for screening but also for risk management. An interesting approach might be radiogenomics, which brings together clinical assessment, imaging results and genetic background [49]. This approach would be of interest in relation to the immunohistochemical staining of the BARD1 gene, which in turn can be imaged in magnetic resonance scans. The precise diagnosis may play a role in decisions about whether to perform or postpone prophylactic surgical interventions due to breast cancer risk. However, the multidirectional diagnostic pathway as a standard approach requires further cohort trials and can be of interest for future researchers.

3.3. BARD1 gene as a potential target of new anticancer therapies including sensitivity to chemotherapy with a focus on breast cancer

Several studies have shown that BARD1 can potentially become a new target for breast cancer treatment. Zhu Y et al. [14] have reported that the significantly higher expression of BARD1 and BRCA1 in tamoxifen-resistant breast cancer cells results in resistance to DNA-damaging chemotherapy with cisplatin and adriamycin, but not with paclitaxel. While the mutations of BRCA1 and BARD1 cause a defective DNA damage repair, they also lead to increased sensitivity to platinum-based chemotherapy. The authors have also suggested that the consideration of microtubule-targeting agents such as taxanes while planning chemotherapy for tamoxifen-resistant breast cancer patients may be superior to DNA-damaging agents (i.e. anthracyclines and platinum compounds). Additionally, they have demonstrated that silencing the gene expression of the aforementioned proteins using siRNAs or phosphorylation inhibition of BRCA1 by a CDK inhibitor, dinaciclib restores the sensitivity to cisplatin in those cells. Since the simultaneous silencing of BARD1 and BRCA1 have failed to show any addictive effect, they have deduced that effects of the therapeutic inhibition are propagated via the same pathway [14]. The same authors have also shown that PI3K inhibitors decrease the expression of BARD1 and BRCA1 in tamoxifen-resistant cells and resensitize them to cisplatin, both *in vitro* and *in vivo*. Hence, they have concluded that the PI3K/Akt/mTOR pathway is responsible for the upregulation of BARD1 and BRCA1. This intracellular signaling pathway is responsible for the control of proliferation, apoptosis, angiogenesis, and cell survival. The

mutations affecting this pathway is the most encountered genetic alteration in ER-positive breast cancer, as well as in recurrent or metastatic cancers [50]. They also increase the activation of the PI3K/Akt/mTOR pathway, which influences the resistance to hormonal cancer therapy [51].

Li M et al. [52] have reported that the BARD1 BRCT domain interacts with poly(ADP-ribose) (PAR), which results in subsequent recruitment of the BARD1-BRCA1 complex to the damaged DNA.

The poly ADP-ribosylation (PARylation) is a particular importance since the promising drugs inhibiting the PAR polymerizing enzyme (PARP) appear to be more efficient in BRCA1-mutated cells with preserved BARD1 tumor suppressor function. The PARylation serves as a signal to recruit DNA damage repair proteins such as the BARD1-BRCA1 complex to the double-strand breaks (DSBs). The BARD1 BRCT domain by binding ADP-ribose, a basic unit of PAR, recruits BRCA1 to the DNA damage sites. This recruitment resulting in formation of BARD1-BRCA1 heterodimer can be suppressed by the PARP inhibition, which selectively eliminate BRCA1-deficient cells. Several PARP inhibitors (PARPi) have recently been approved by the FDA for the treatment of various neoplasms, including metastatic TNBC and estrogen receptor-negative (ER-)/HER2+ breast cancer with BRCA mutations [53].

Throughout treatment both ovarian and breast cancer patients harboring BRCA1 mutations initially responding to the platinum and PARPi therapy, develop the resistance to both PARPi and platinum compounds [54,55,56]. This resistance, as examined by the patient's biopsies could result from the observed secondary mutations or the methylation status of BRCA1, BRCA2 and other genes controlling the homologous recombination. One potential way to overcome this resistance could be the investigation of whether the expression of FL or the isoform of BARD1 contributes to the success or failure of the PARPi therapy [54].

None of above-mentioned inhibitors specifically affect BARD1. Lepore et al. [57] have shown that Vorinostat, a histone deacetylase inhibitor (HDACi) lowers the BARD1 isoform mRNA revels through increased miR-19a and miR-19b expression. Additionally, they have reported that the expression of the truncated BARD1 isoforms expressed in human acute myeloid leukemia (AML) cell lines is modulated by HDACi treatment via miR-19a/b. To verify whether this was an exclusive event to human AML cell lines, they have evaluated Vorinostat-induced downregulation of BARD1 expression in additional human cancer cell lines, such as MCF7 breast cancer cells, HeLa cervical cancer cells, and Kelly neuroblastoma cells. The time-dependent reduction has been observed in Kelly and MCF7 cell lines, but not in HeLa lines, indicating that the BARD1 dysregulation is cell line-restricted. Interestingly, cells affected by Vorinostat weakly express FL BARD1 [57].

3.4. BARD1 gene alterations in neoadjuvant setting in breast cancer

Current data on this subject mainly refer to TNBC which has higher incidence of pathogenic variants of the BARD1 gene [58]. Watanabe et al. analyzed thirty TNBC core biopsy specimens of patients with pathologic complete response (non-invasive cancer) and non-complete response following neoadjuvant chemotherapy (NACT), with regard to the aberrant DNA methylation status of the BARD1 gene (from a total number of 16 DNA repair genes) using bisulfite-pyrosequencing. Although hypermethylation of BRCA1 gene is associated with TNBC subtype and may impact chemosensitivity and progression under NACT, BARD1 gene hypermethylation revealed only a low-to-moderate influence on these processes [59]. Some other studies underline the low incidence and uncertain clinical impact of gene mutations other than BRCA1/2 (including BARD1), and the associated unfavorable outcomes for patients with breast cancer undergoing NACT [60]. Yet other studies reported that BRCA1 and its associated protein BARD1 are upregulated in tamoxifen-resistant breast cancer cells, rendering the cells resistant to DNA-damaging chemotherapy [14,61]. Today, neoadjuvant chemotherapy makes a significant contribution to chemotherapy in breast cancer and is a bridge towards adjuvant regimens and other therapies. Intensifying research into the role of BARD1 in chemotherapy in women for whom NACT is planned is essential and should be of benefit.

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4. Discussion

The U.S. National Comprehensive Cancer Network (NCCN) guidelines do not routinely recommend BARD1 positive-patients to undergo additional breast cancer screening (early breast MRI, mammography), which might need to be implemented [21]. This screening is usually only performed in the cases with family history indicating that the patient has an increased risk of breast cancer. For now, the risks of breast cancer connected with BARD1 remain poorly defined and of varying prevalence across different populations. Nonetheless, there is multiple instances, listed in the evidence above, that the PVs of BARD1 not only increase the risk of breast cancer in general, but primarily of TNBC, and can be associated with age at first diagnosis of equal or under 50 years.

The BARD1 gene can significantly extend the monitoring options in patients at risk of breast cancer and during post-treatment follow-up. However, due to the low incidence of BARD1 mutations, such assumptions require further long-term population-based trials. At present, and considering potential benefits and costs, it seems that the possible uses of the BARD1 gene that we discussed in this review can set a direction for further research rather than provide real options for widespread use. However, it should be noted that commercial tests are available that can detect a mutation in the BARD1 gene.

Over the last few decades, molecular research has been intensified to further individualize the treatment of breast cancer patients. Personalization of systemic treatment is aimed at identifying a group of patients with unfavorable prognostic factors and at identifying patients who can benefit most from therapy [47]. The assessment of efficacy of PARPi in breast cancer patients with the relatively frequent LoF mutations of BARD1 would be of necessity to improve patients' outcomes. Ozden et al. [62] proved that BARD1β sensitizes colon cancer cells to poly PARP-1 inhibition even in an FL BARD1 background, thus suggesting that BARD1β may serve as a future biomarker for assessing the suitability of colon cancers for homologous recombination targeting with PARPi in the treatment of advanced colon cancer. Clinical trials of PARPi in neoadjuvant, mono- and combination therapy settings in breast cancer are ongoing.

Neoadjuvant chemotherapy offers opportunity to assess the molecular changes of heterogenic breast cancer tissue before and after chemotherapy, especially in the case of TNBC, in which BARD1 gene deleterious alterations are the most prevalent and NACT seem to have the greatest value.

Near half of cited papers about breast cancer in this review did not relate to any molecular subtype. Most of the relevant studies suggesting the role of BARD1 in breast cancer is in TNBC patients. This shows that more research is based and needed into the genesis and therapeutic potential of TNBC.

5. Conclusions

Analyzing structure and functions of the BARD1 gene, we believe that BARD1 gene can play an important role in the pathogenesis of breast cancer and in the mechanisms of chemo-resistance of cancer cells as well.

It is reasonable to screen BARD1 gene isoforms in certain populations, especially in those with evidence of higher prevalence of mutations in the BARD1 gene. This approach would also have to be researched for its relevance to general breast cancer patient outcomes, survival rates, quality of life, influence on treatment decisions and cost-effectiveness.

Radiogenomics is a promising field of science as a bridge between molecular and imaging medicine. Broader prospective studies and standardization (i.e. immunohistochemistry studies with BARD1-directed antibodies) will provide determination of appropriate imaging biomarkers enabling "cancer cell visibility" before they can be introduced in to a clinical investigation.

Further research on the BARD 1 gene expression may contribute to the effective reversal of PARPi resistance and the wider introduction of new targeted therapies for the treatment of breast cancer patients.

Data on patients with BARD1 gene polymorphism undergoing NACT for breast cancer are limited. However, gene expression alterations after NACT can shed light on the pathogenesis of this multifactorial disease.

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